1808-118
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
JAMES SCOTT CROWE et al.	
Serial No. 07/952,640	Examiner: C. Eisenschenk
Filed: December 1, 1992	Group Art Unit: 1806
For: PRODUCTION OF ANTIBODIES	

DECLARATION

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Alan Peter Lewis, declare that:

1. I am the same Alan Lewis named as an inventor on the above-referenced patent application.

2. I received my B.Sc. Honors Degree in Biological Sciences in 1984 and my Ph.D in 1987 from the University of Leicester. From 1987-1990 I conducted postdoctoral research in molecular biology at Wellcome Biotech. Since 1990, I have been employed as a molecular biologist in the department of cell biology at the Wellcome Research Laboratories. In this position, my work has focused on carrying out fundamental research in immunochemistry using recombinant DNA technology for the purpose of formulating new therapeutic antibody molecules. A copy of my curriculum vitae is attached.

3. This application is a continuation of earlier-filed application Serial Number 07/952,640. The application discloses and claims a process for the production of a recombinant primate antibody. The process of the invention comprises selecting a cell line derived from a primate lymphocyte that is capable of expressing a desired antibody, isolating RNA from that cell line and separating mRNA from the other isolated RNA, synthesizing cDNA from the mRNA and inserting that cDNA into a cloning vector, transforming a host cell with the vector containing the cDNA in order to obtain a library, screening the library for cDNA which encodes the entire constant and variable regions of both the heavy and light chains of the desired antibody, inserting that cDNA into an expression vector, culturing the transfected host cell under antibody-producing conditions and then isolating the desired antibody.

4. I have carefully reviewed the May 27, 1994, Office Action and the references cited therein and the Advisory Action of November 29, 1994, issued in connection with the parent application. As a result of my careful review, it is my opinion that the references cited, taken either alone or in combination, do not render the invention obvious to persons of ordinary skill in the art for the following reasons.

5. In the Advisory Action, the rejection of claims 1-2, 4-5, 7-10, and 12-14 as obvious under 35 U.S.C. § 103 in view of the teachings of Cillies et al. was maintained. In the May 27, 1994, Office Action, the Examiner had asserted that the claimed

invention was obvious because the specification indicates that methods of inserting complete cDNA sequences into expression vectors were known prior to the present invention, citing page 14, second paragraph, of the specification in support. In the Advisory Action he further stated that there was no showing that the claimed method produces unobvious results in comparison to the method of the prior art.

6. With regard to this § 103 rejection, the paragraph of the specification cited by the examiner provides that heavy and light chain cDNA can be transfected in a single vector as taught in PCT application WO 87/04462 or co-transfected in two vectors as subsequently taught in Applicants' invention. The 04462 application discloses expression vectors containing a recombinant DNA sequence encoding the complete amino acid sequence of a glutamine synthetase. More specifically, the application teaches a method for co-amplifying a recombinant DNA sequence which encodes a desired protein by either (1) co-transforming a host cell with a vector containing a DNA sequence encoding a glutamine synthetase and a vector comprising a recombinant DNA sequence which encodes the desired protein or (2) transforming a host cell with a vector containing a DNA sequence encoding a glutamine synthetase and which further contains a recombinant DNA sequence encoding the desired protein. Expression of the glutamine synthetase gene provides transformant cells with a dominant selectable marker; cells transformed with the two vectors have resistance to glutamine synthetase inhibitors. The focus of the

04462 application is on providing the recombinant DNA encoding the complete amino acid sequence of a glutamine synthetase and its use in the construction of expression vectors. There is very little discussion in the application about the DNA encoding the desired protein. In the paragraph bridging pages 9-10 of the 04462 application, the applicants state that this DNA can encode tissue plasminogen activator (tPA) or "any other protein, such as immunoglobulin polypeptides (IGs), human growth hormone (hGH) or tissue inhibitor of metalloproteinases (TIMP)." There is no other discussion about the DNA encoding the protein of interest and no discussion of the insertion of complete sequences of both the heavy and light chains of a desired immunoglobulin. There is no discussion in the reference whether the reference to "DNA" is intended to refer to cDNA, genomic DNA or a combination of the two. There is no teaching that the light and heavy chains of an antibody could be provided by expressing cDNA encoding the variable and constant regions of each chain. Therefore, contrary to the examiner's assertion, this reference does not provide an enabling disclosure that antibodies can be produced by inserting the cDNA encoding both the constant and variable regions of the antibody light and heavy chains into an expression vector under the control of expression signals, transfecting a host cell with the vector and then culturing the host under protein-producing conditions.

7. Gillies et al. teach the expression of a human antibody gene in a transfected murine myeloma cell line. In

describing the construction of the expression vectors, they teach that the polyadenylation site of the cDNA encoding immunoglobulin chain polypeptides should be removed. This teaching was based upon what had been taught in the art at the time this paper was written as explained below.

In discussing the state of the art at the time of the Gillies paper, reference will be made to the drawings provided as Attachment A to this declaration. Figure 1 of Attachment A provides an illustration of genes encoding each of the light and heavy chains of an antibody. As shown in Figure 1, the 3' end of the heavy chain comprises a poly A sequence followed by a consensus sequence. When messenger RNA is generated for either the light or heavy chain, the formation of the mRNA 3' end is controlled by the consensus sequence located a specified distance downstream of the poly A site; the mRNA will form incorrectly and not be translatable into protein if the consensus region is not present. The mRNA generated for each chain stops just after the poly A sequence; the cDNA obtained from the mRNA also contains the poly A sequence but not the downstream consensus region. The insertion of such a sequence into a vector that has its own poly A sequence and consensus region would result in a DNA sequence resembling that of Figure 2 of Attachment A. Absence of the consensus sequence at the correct distance from the first poly-A site would be expected to result in incorrectly formed inactive mRNA. Accordingly, at the time Gillies did his experimental work it was believed important to remove the poly A sequence of the

antibody heavy and light chain genes prior to expressing them. In addition, it was believed desirable to also remove instability sequences present in the 3' untranslated regions of DNA to ensure efficient high level expression from genes, as these sequences render mRNA unstable.

For the sequence encoding the light chain of an antibody, a restriction enzyme can be used in a partial digest to remove the poly A sequence as well as the preceding 3' untranslated sequences in the constant region DNA. Gillies thus was able to use cDNA encoding the variable and constant regions of the light chain of the desired antibody in his expression vector. For the constant region of the heavy chain, however, no appropriate restriction enzyme sites exist to allow cleavage of the sequence to remove the 3' untranslated sequence and the poly A site. Accordingly, Gillies grafted genomic DNA encoding the heavy chain constant region to the cDNA encoding the variable region of the heavy chain of the antibody of interest.

8. A person of ordinary skill in the art who had read both the Gillies reference and the 04462 application would be left with the conclusion that if one wished to produce a recombinant antibody, one would need to remove the 3' untranslated region of each sequence. For the heavy chain, this could only be achieved by removing the region encoding the constant region and replacing it with genomic DNA of the Ig class. For the light chain, this could be achieved by subjecting the sequence to the action of a restriction enzyme which would partially digest the sequence so

as to remove the 3' end of the sequence including the poly A sequence. In contrast, with the present process we went against the teachings of the prior art and found that, surprisingly, the entire cDNA, including the polyadenylation sequence and preceding untranslated region, was suitable for human immunoglobulin expression with no further processing required, and without the necessity for genomic DNA sequences. Thus, claims 1-2, 4-5, 7-10 and 12-16 are not rendered obvious by Gillies et al. and WP 87/04462.

9. Claims 3 and 6 were rejected under 35 U.S.C. § 103 as unpatentable over Gillies et al. in view of Foung et al. and Ehrlich et al. In maintaining this rejection, the examiner reiterated his earlier comments regarding the Gillies reference and PCT WO/04462.

10. I believe that the above comments regarding the deficiencies of the Gillies et al. reference and the 04462 application are equally valid in connection with the rejection of claims 3 and 6. Gillies et al. teach using a combination of cDNA and genomic DNA to provide a DNA sequence encoding the constant region of the heavy chain of an antibody and a partially digested cDNA sequence encoding the light chain. In contrast to this teaching, Applicants use cDNA encoding the entire constant region and the entire variable region for each of the heavy and light chains of the antibody. The 04462 reference, as explained above, does not teach that using cDNA sequences encoding the entire heavy and light chains of an antibody was known prior to the

present invention. The two secondary references do not overcome the deficiencies of the Gillies et al. paper or the 04462 application. Neither Foung et al. nor Ehrlich et al. teach or suggest any method for producing recombinant antibodies, much less that a recombinant antibody could be produced using the cDNA encoding the entire constant and variable regions of each of the heavy and light chain of the antibody. Thus claims 3 and 6 are not rendered obvious by the references alone or in combination.

11. Claim 11 stands rejected under 35 U.S.C. § 103 as obvious over Gillies et al. in view of Larrick et al. The secondary reference does not compensate for the deficiencies of the primary reference. A person of ordinary skill in the art who had read these two references would not be motivated to construct an expression vector in which the DNA encoding the constant and variable regions of each chain of the antibody of interest are provided by cDNA. The Larrick et al. reference teaches a method of amplifying monoclonal antibody variable region DNA using PCR. The method does not provide for the amplification and cloning of the entire gene including both constant and variable regions.

12. For the reasons stated above, there is no motivation provided by the prior art to develop the method of this invention and, absent hindsight, no expectation of success for the invention as claimed.

13. In addition, I would like to point out that the present process has two major advantages over the prior art processes: (1) simplicity resulting in savings of time, cost, and effort;

and (2) ability to rescue human antibodies which have been screened for antigen specificity and effector function. The prior art processes, which teach that the cDNA sequence of the heavy chain should be expressed with genomic DNA replacing a portion of the constant region and that the cDNA sequence encoding the light chain should be partially digested with a restriction enzyme to remove the polyadenylation site and 3' untranslated region, are more difficult and costly to perform. In addition, the outcome may be an inferior antibody, which does not contain the entire constant region of the native antibody and therefore does not necessarily possess the same effector function which is necessary for the most efficient use of the antibody as a diagnostic aid or as a therapeutic agent.

14. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or

imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Alan Lewis

28th September 1995
Date

\1808-118.DC3

ATTACHMENT A

Figure 1

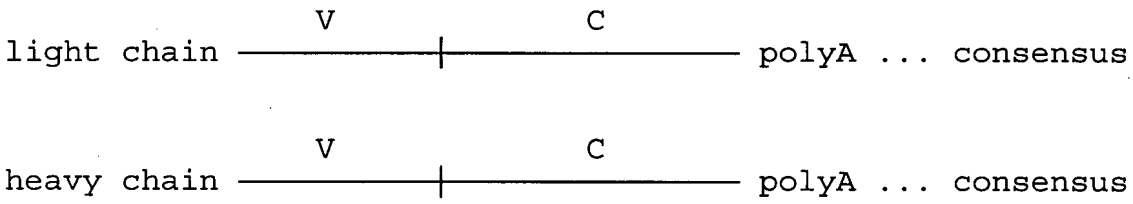
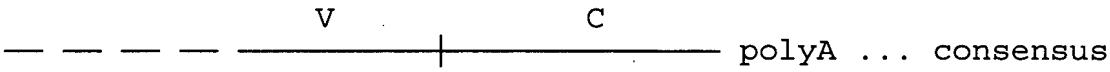


Figure 2



CURRICULUM VITAE

Name: Alan Peter LEWIS

Address: 20 Morgan Road
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Telephone no: 081 466 6914

Date of Birth: 11th February 1963

Nationality: British

Marital Status: Married

First Degree:

1981 - 1984 University of Leicester

B.Sc. Honours Degree in Biological Sciences, Grade IIi

2nd Year Course Units: Genetics Systems; Molecular Genetics; Genes and Development I; Basic Microbiology; Biology of Non-Chordates; History of Biology.

3rd Year Course Units: Current Topics in Molecular Genetics; Genes and Development II; Animal Cytology; Biochemistry of Gene Expression.

3rd Year Project: "The use of spot hybridisation techniques to search for an *Escherichia coli gyrB*-like DNA sequence in the genomes of various eukaryotic and prokaryotic organisms".

Postgraduate qualifications:

1984 - 1987 University of Leicester, Faculty of Medicine

Ph.D.: "The characterisation of P element-induced singed mutations in *Drosophila melanogaster*, and an analysis of the extent of mobilisation of transposable elements in a P-M hybrid dysgenic cross".

Postdoctoral research:

1987 - 1990

Postdoctoral molecular biologist, Grade 8
Department of Molecular Biology
Wellcome Biotech
Langley Court
Beckenham
Kent, BR3 3BS

Project funded by the World Health Organisation:
"An animal model (*Plasmodium yoelii*) for the development of a vaccine against the asexual blood stage of *Plasmodium falciparum* based on the precursor to the major merozoite surface antigens".

1990 -

Molecular Biologist (presently Grade 10)
Department of Cell Biology
Wellcome Research Laboratories
Langley Court
Beckenham
Kent, BR3 3BS

The major objective of the work is to perform fundamental research in immunochemistry using recombinant DNA technology for the purpose of formulating new therapeutic antibody molecules. In particular, this involves the creation of novel humanised monoclonal antibodies and the rescue of human monoclonal antibodies.

Publication List: See separate sheet.

Interests, hobbies and recreations:

I play the alto saxophone, clarinet, flute, piano and keyboards, having performed in jazz-rock and session groups, and in a number of big bands, including "The Welsh Jazz Orchestra". I enjoy writing and arranging classical, jazz and rock music.

I take an active part in most sports and play badminton and football for the Wellcome (Beckenham) Sports and Social Club.

Full driving licence obtained in February, 1981.

I am a member of British Mensa.

Research Studies:

Postgraduate research

Ph.D.: "The characterisation of P element-induced singed mutations in *Drosophila melanogaster*, and an analysis of the extent of mobilisation of transposable elements in a P-M hybrid dysgenic cross".

The *Drosophila melanogaster* transposable element, P, is linked to the process of P-M hybrid dysgenesis, a syndrome of abnormalities observed in the offspring of a cross between a male of a P factor-containing strain and a female of a strain lacking the elements. The abnormalities are the result of elevated P transposition rates in such a cross.

There has been speculation as to whether other transposable element families are also mobilised during P-M hybrid dysgenesis. Isofemal lines derived from such a cross were analysed by *in situ* hybridisation using cloned copies of the transposable elements *copia*, *412* and F. It was found that lines derived from dysgenic crosses showed a statistically significant number of new sites for these elements when compared to a non-dysgenic control. This result suggests a functional coupling of *copia*, *412*, and F transposition and some component present in the P-M dysgenic system.

The singed locus is a "hotspot" for P element insertions during P-M hybrid dysgenesis. A number of sn mutations have been induced by this process, which possess different properties in terms of their phenotypic strengths, germline reversion rates, and somatic destabilisation by a certain trans-acting component. Characterisation by Southern and Northern blotting revealed that in each mutant strain the elements were inserted within the 5' transcribed regions of the gene causing a reduction in level of sn poly $(A)^+$ RNA, and also, in one case, the production of two novel mRNA species. It was also determined that the germline and somatic reversion frequencies of the strains were most probably dependent upon the target sites into which the elements were inserted. Repeated attempts to clone two insertions at the sn locus in one of the strains proved unsuccessful, most probably due to the unusual structure exhibited by the elements in this strain.

Postdoctoral research:

"An animal model (*Plasmodium yoelii*) for the development of a vaccine against the against the asexual blood stage of *P.falciparum* based on the precursor to the major merozoite surface antigens".

The gene encoding the 230kDa precursor to the major merozoite surface antigens (PMMSA) of *Plasmodium yoelii* YM has been c cloned, and the complete nucleotide sequence determined. A single open reading frame of 5316 base pairs encodes a polypeptide of calculated molecular mass 197232. The deduced amino

acid sequence contains potential signal peptide and membrane anchor sequences of 19 and 18 residues respectively, and a region of six tandemly repeated tetrapeptides, Gly-Ala-Val-Pro. There are 20 cysteine residues and 11 potential N-glycosylation sites.

Computer analysis was used to compare the amino acid sequence of the *P.yoelii* YM Py230 antigen with the complete sequences of the two Pf195 alleles from the *P.falciparum* Wellcome and MAD20 strains, and also with a published region of the *P.vivax* PMMSA, Pv200. Certain regions of the PMMSA were found to be conserved between the three evolutionary distant malarial species, thus arguing for some important structural and/or functional constraints in these regions. Extensive homologies were found throughout the length of the molecule thus reinforcing the use of Py230 as a rodent model for the PMMSA. The polypeptide was divided into 22 blocks classed as either conserved, semi-conserved or variable, based upon amino acid conservation.

A fragment from the Py230 gene was used to probe an RNA Northern blot of total insertion of sequences from the signal peptide region of a baculovirus capsid protein gp67 before the cloning site. Py230 gene fragments analogous to the regions encoding the Pf195 processing fragments were PCR amplified and cloned into the expression vectors. Py230 regions analogous to the Pf195 42, 38 and 83kDa polypeptides, cloned into a vector possessing just the gp67 signal sequence, were expressed in the baculovirus/insect cells system. The protein products were detected on Western blots using monoclonal antibodies McAb302 and Mab 25.1, which recognise the epitopes within the carboxy- and amino-terminal portions of the Py230 respectively, and rabbit polyclonal Py230 antiserum. The Pf42 and Pf38 analogous polypeptides were found to be situated in cell pellet fractions after centrifugation, whereas the polypeptide analogous to Pf83 was secreted into the culture medium, thus suggesting that sequences immediately downstream of the signal peptide cleavage site may be important for correct secretion. A second vector has thus been constructed possessing the gp67 signal sequence plus an additional 10 downstream amino acids, into which Py230 fragments have been cloned.

The Py230 region analogous to Pf83 was expressed in insect cells and purified from the culture medium using Mab 25.1-Sepharose 4B affinity chromatography. Animal experiments are presently underway in collaboration with C. Long (Hahnemann University, Philadelphia).

Present research:

My present work involves the formulation of novel therapeutic monoclonal antibodies (mAbs), especially in the areas of inflammation and anti-viral therapy, using molecular biological gene manipulation and eukaryotic gene expression techniques. The use of rodent mAbs as human therapeutics has been hampered by the failure of most rodent Ab isotypes to activate human effector mechanisms, and by the immunogenicity of rodent mAbs in humans. This has led to the humanisation of mAbs, that is, the transplantation of the

complementarity-determining regions from the appropriate rodent mAb onto a human immunoglobulin (Ig) framework. I have developed and patented a new technique for mAb humanisation utilising the recombinant polymerase chain reaction. This method has been used to humanise a rat anti-interleukin 2 receptor mAb which may have uses in transplant and autoimmune disease therapy.

Human mAbs would be preferable to rodent mAbs as therapeutic agents. However, although human mAbs have been produced by conventional hybridoma technology and by EBV transformation the general production of such reagents has been dogged by instability of the cell lines and low levels of expression of the mAbs. I have been involved with the development and patenting of a method allowing the rescue of human Igs enabling the immortalisation of functional human mAbs. This technique has been used to rescue an anti-hepatitis A human mAb.

Research is also under progress investigating the use of monkey mAbs as therapeutics.

PUBLICATION LIST
Alan P. Lewis

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Brookfield, J.F.Y. and Lewis, A.P. (1989) Somatic reversion of P transposable element insertion mutations in the *singed* locus of *Drosophila melanogaster* requiring specific P insertions and a *trans*-acting factor. Genet. Res., Camb. 54:101-112.

Lewis, A.P. (1989) The 230000 molecular mass merozoite surface antigen of *Plasmodium yoelii*: cloning and analysis of the 3' half of the gene. J. Cell. Biochem. 13E:142.

Lewis, A.P. (1989) Cloning and analysis of the gene encoding the 230-kilodalton merozoite surface antigen of *Plasmodium yoelii*. Mol. Biochem. Parasitol. 36:271-282.

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Lewis, A.P. and Crowe, J.S. (1991) Immunoglobulin complementarity-determining region grafting by recombinant polymerase chain reaction to generate humanised monoclonal antibodies. Gene 101:297-302.

Lewis, A.P., Parry, N., Peakman, T.C. and Crowe, J.S. (1992) Rescue and expression of human immunoglobulin genes to generate functional human monoclonal antibodies. Hum. Antibod. Hybridomas. 3:146-152.

Gewert, D., Salom, C., Barber, K., Macbride, S., Cooper, H., Lewis, A., Wood, J. and Crowe, S. (1993) Analysis of interferon-α2 sequences in human genomic DNA. J. Interferon Res. 13:227-231.

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Lewis, A.P., Lemon, S.M., Barber, K.A., Murphy, P., Parry, N.R., Peakman, T.C., Sims, M.J., Worden, J. and Crowe, J.S. (1993) Rescue, expression, and analysis of a neutralizing human anti-hepatitis A virus monoclonal antibody. J. Immunol. 151:2829-2838.

Lewis, A.P., Barber, K.A., Cooper, H.J., Sims, M.J., Worden, J. and Crowe, J.S. (1993) Cloning and sequence analysis of κ and γ cynomolgus monkey immunoglobulin cDNAs. Developmental Comp. Immunol. 17:549-560.

Crowe, J.S., Gewert, D.R, Barber, K.A., Lewis, A.P., Sims, M.J., Davies, S.L., Salom, C.L., Wood, J., Thomas, H.C., Thursz, M. and Lok, A.S. (1994) Interferon (IFN)-α2 genotype analysis of Chinese chronic hepatitis B patients undergoing recombinant IFN-α2a therapy. J. Infect. Dis. 169:875-878.

Lewis, A.P., Sims, M.J., Gewert, D.R., Peakman, T.C., Spence, H. and Crowe, J.S. (1994) *Taq* polymerase extension of internal primers blocks polymerase chain reactions allowing differential amplification of molecules with identical 5' and 3' ends. Nucleic Acids Res. 22:2859-2861.